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REPORT ON INVESTIGATION OF FISH KILLS IN LOWER MISSISSIPPI RIVER, ATCHAFALAYA RIVER, AND GULF OF MEXICO

Public Health Service
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U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

Public Health Service

Division of Water Supply and Pollution Control

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PREFACE

On November 18, 1963, Mr. Robert F. LaFleur, Chief, Division of Water Pollution Control, Louisiana State Wildlife and Fisheries Commission, asked the Public Health Service's Division of Water Supply and Pollution Control to investigate massive fish kills in the lower Mississippi and Atchafalaya Rivers and the Gulf of Mexico. These kills have taken place each year since 1960 during the fall and winter months and were particularly severe this year. A search for the cause of the kill by Louisiana's Division of Water Pollution Control had proved fruitless. A number of theories had been advanced including cumulative pesticide poisoning and bacterial disease. Although evidence could be cited to support various theories, none of them had been substantiated with scientific proof.

In response to the request for technical assistance, the Division of Water Supply and Pollution Control sent a team of investigators to the lower Mississippi River area early in December 1963. These field investigations have been supported by work at the Robert A. Taft Sanitary Engineering Center, Cincinnati, and the Division's Pesticide Pollution Laboratory, Atlanta. Three of the Division's Branches have been involved in this study in cooperation with the State of Louisiana; Basic Data, Basic and Applied Sciences, and Technical Services. The Division of Environmental Engineering and Food Protection has also participated in these investigations. Personnel of the Fish and Wildlife Service, U. S. Department of the Interior, have cooperated in investigating some of the relationships of the problem to fish diseases.

The purpose of this report is to set forth the initial findings of the investigations.

SUMMARY AND CONCLUSIONS

1. Massive kills of fish have occurred in the lower Mississippi and Atchafalaya Rivers and the Gulf of Mexico, in the fall and winter months every year since 1960, and were particularly severe in the winter of 1963-64. Rough estimates of the size of these kills have been as follows: 1960, 3.6 million fresh and salt water fish; 1961, 275,000; 1962, 275,000; 1963, 5.2 million. The kills have taken place sporadically over a period of several months, with no apparent pattern as to place of individual kills. There have been unconfirmed reports of dying fish as far north as St. Louis, and kills have extended downstream to salt water.

In the fresh waters, primarily bottom-feeding fish were affected; while in the brackish waters bottom- and surface-feeding species were involved. Channel catfish, the principal species affected in fresh water, is commercially important in the lower Mississippi area. There are reports of dead and dying catfish being collected and sold for human consumption.

2. In response to a request for technical assistance from the Chief, Division of Water Pollution Control, Louisiana State Wildlife and Fisheries Commission, the Public Health Service, Division of Water Supply and Pollution Control, initiated an investigation into the cause of the kills.

3. Public Health Service scientists examined various possible causative factors. The kills could not be related to river flows, and adverse environmental conditions due to pollution, such as oxygen depletion or temperature increases, were ruled out. Bacterial or viral diseases, although suspected, were eliminated with the help

of the Fish and Wildlife Service, U. S. Department of the Interior.

Autopsies of fish revealed that toxic substances were involved, but it was found that the kills were not due to metals or organic phosphorous insecticides.

4. A rigorous examination of the tissues of dead and dying fish using recently developed and highly sensitive analytical methods, revealed the presence of endrin, dieldrin, DDT, DDE and other synthetic organic chemicals. Endrin was consistently found in all tissue extracts examined.

5. Experiments conducted at the Sanitary Engineering Center in Cincinnati, showed that endrin was present in lethal amounts in the blood of dead fish taken from the Mississippi River. The symptoms of the fish killed experimentally were identical to those of dying fish from Louisiana. As a result of these experiments and related investigations, endrin appears to have been the most likely cause of the fish kills in 1963. Because of the many similarities involved, it is quite probable that the kills in earlier years were caused by the same chemical.

6. Specimens of dead or moribund catfish collected from the Mississippi River at Baton Rouge in December 1963 were found to be toxic to mice in tests conducted by the Division of Environmental Engineering and Food Protection.

Similar tests with oysters taken in good condition from Grand, Quarantine, American Bays and other shellfish growing areas were not found to be toxic.

7. During the fall and winter migratory season in 1963, there have been periodic observations in Louisiana of individual ducks dropping from flying flocks. One such duck was examined and found to contain

endrin, dieldrin, DDT, DDE and other synthetic organics. Living shrimp collected from the delta of the Atchafalaya River were found to contain these substances as well as other insecticides. No reports of shrimp mortality have been received.

8. Carbon filter samples collected routinely from a number of points along the Mississippi River during the summer and fall of 1963, as a part of the Public Health Service's nationwide water pollution surveillance system showed the presence of endrin and dieldrin. Other samples of Mississippi River water collected in Missouri and Illinois in December 1963 also contained dieldrin.

9. Raw water samples collected from the Mississippi River during December 1963 and January 1964 were submitted to bioassay using brine shrimp and a small brackish-water fish, Mollenisia laptima and found to be toxic. Samples of water from several marine areas tributary to the Mississippi were tested and found to be non-toxic.

10. Samples of treated water from the New Orleans public water supply system have also been found to contain endrin. A carbon filter extract showed that there was an average concentration of endrin of at least 0.160 parts per billion during the period July 28 - August 13, 1963. This is believed to be the highest average concentration of endrin observed in a finished water supply.

11. Although the findings have been highly significant with respect to the cause of the fish kills, they have also highlighted the complex and difficult nature of the Lower Mississippi problem. The current investigations represent merely a start in terms of developing a program of prevention and control. It is not yet known how the contaminants reach the watercourses or precisely how they are concentrated in fish. Further, this area received the drainage from a major

portion of the United States, and wastes from a myriad of sources contribute to pollution of the lower Mississippi

12. On the basis of the findings to date, the Secretary of Health, Education, and Welfare has called a four-State enforcement conference under the provisions of the Federal Water Pollution Control Act. The purpose of the conference will be to seek possible remedial measures which can be taken at the present time, and to delineate the needs for further information. The Public Health Service proposes to initiate an over-all and intensified water pollution control study in cooperation with the States and other Federal agencies and industries concerned, to obtain the information necessary to develop both immediate and long-range remedial measures.

HISTORY OF THE FISH KILLS

Discussion with Mr. LaFleur and a search of records revealed a seasonal pattern of fish kills in the Louisiana area. The first report of fish die-off came from the Atchafalaya River on November 17, 1960, near Krotz Springs, Louisiana. This mortality extended as far downstream as Morgan City. Fish were neither reported nor observed dying in the Atchafalaya drainage (Red and Black Rivers) above the Old River connection with the Mississippi River. Shortly thereafter, fish mortalities were reported in the Mississippi River as far upstream as Lake Providence (mile 480 at head of passes) and downstream to Venice (mile 10 at head of passes). The greatest numbers of fish dying were observed in the back waters of the Atchafalaya such as Bayou Sorrel, Six Mile and Grand Lake.

Species of fish affected included catfish, carp, fresh water drum, and threadfin shad. Approximately 95% of the fish dying were catfish. In addition, most of the catfish dead and dying in the Bayou Sorrel area were thought to be from the 1960 hatch. These fish were from six to eight inches in length and weighed from one-half to three-fourths of a pound. However, a few large individuals weighing 25 pounds or more were noted.

Dissolved oxygen and pH determinations were made at all locations where dead or dying fish were found in 1960 by personnel of the Louisiana Division of Water Pollution Control. In all cases the pH fell within the range from 6.8 to 8.2 and the dissolved oxygen content was in excess of 7 ppm and approaching saturation for the prevailing temperatures.

Repeated observations of dead or dying fish revealed several rather constant phenomena. Most of the catfish were noted to be hemorrhaging around the mouth and many were hemorrhaging about the fins, particularly

the pectoral fins. In every instance, the catfish were observed to have a distended abdominal area. Gross examination revealed that the distention was observed in the air bladder and digestive tract. The latter was devoid of food material and contained only gas or air and a very small amount of frothy material. Dying fish were swimming at the surface (often inverted) in a very lethargic manner and were easily captured by hand. However, even though they were at the surface, the fish exhibited no gulping actions as are noted in case of respiratory difficulty. Another feature noted was belching of large quantities of air or gas by some of the catfish.

During the investigation of the 1960 fish kills repeated attempts were made to bring affected fish into the laboratory to expose healthy fish from another area where no fish mortality had occurred. Of the numerous fish brought to the laboratory only four individuals survived for more than a few hours, and these four recovered completely. Two healthy fish held in the tank with these four individuals showed no ill effects.

Extraction of organic compounds with carbon tetrachloride were made from bottom muds in the affected areas and the extracts subjected to infrared and paper chromatography analyses. The findings offered no clues to possible causes of mortality.

Mud samples and fresh water clams were sent to the Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio, for analysis and possible identification of pesticides. Results were negative with respect to pesticides. Dead and dying fish were frozen and shipped to Dr. S. F. Snieszko, Microbiological Laboratory, U. S. Fish and Wildlife Service,

Kearneysville, West Virginia. In addition, fish, clams, and mud samples were submitted to the Patuxent Research Center, U. S. Fish and Wildlife Service, Laurel, Maryland, through Dr. L. L. Glasgow of Louisiana State University. Results were negative with respect to pathogenic bacteria or other reasons for the fish kill. The Louisiana Division of Water Pollution Control attempted to culture bacteria from parts of the viscera of affected fish without success.

A seasonal kill again occurred in 1961 and 1962. Louisiana officials observed that the fish died during the early winter months. Mortality seemed to occur when water temperatures dropped below 15° - 20° C. Even then, the kill was not continuous or complete. It was described as intermittent, following no pattern that could be correlated with hydrology or environmental conditions other than a gradual temperature change. However, a pattern did develop in the fresh and salt water species affected. In the fresh waters, primarily bottom feeding fish were affected; while in the brackish waters of the Gulf, bottom and surface feeders were involved.

The kills in 1961 and 1962 were not as severe as those of either 1960 or 1963. However, in all four years the same general pattern was noted except that a larger variety of species were found dead or dying in the years following 1960. Catfish, drum, buffalo and shad were observed swimming near the surface of the river and dying. In brackish waters near the Gulf of Mexico, whole schools of menhaden and numerous mullet, sea trout and marine catfish died.

Each year kills occurred at the same time in both the Atchafalaya River and the Mississippi Channel.

Mr. LaFleur stated that it was extremely difficult to estimate the number of fish killed because there was good reason to believe that only a small portion of the die-off has actually been observed. The following table is his best estimate of the number of fish killed:

Year	Mississippi		Atchafalaya
	Fresh Water	Salt Water	Fresh Water
1960	100,000	1,000,000	2,500,000
1961	25,000	200,000	50,000
1962	25,000	200,000	50,000
1963	75,000	5,000,000	100,000

OBSERVATIONS ON THE 1963 FISH KILLS

The first mortality in the winter of 1963-64 was observed in November 1963, somewhat earlier than in the previous years. Two research scientists from the Basic and Applied Sciences Branch, Division of Water Supply and Pollution Control, visited the lower Mississippi River during the first week in December. They made on-site investigations and collected specimens and samples for laboratory study. The following description of the 1963-64 fish kills is based upon their observations and upon information in the files of the State of Louisiana's Division of Water Pollution Control. It should be emphasized that knowledge of the full extent and duration of the kills is incomplete. The kills occurred over such a large area that it is impossible to describe accurately the sequence of events or the extent of the kills. The density of dying fish frequently was not great, and thus many of the kills might have escaped detection.

Reports coming to Mr. LaFleur indicated that the kill extended as far north as St. Louis, Missouri, and south into the brackish water areas

at the mouth of the Mississippi River. The kills to the north were reported by tug boat crews and as yet have been unconfirmed by water pollution control officials in Kentucky, Mississippi, Missouri, or Tennessee.

The kill consisted mainly of catfish, drum, buffalo, and shad in fresh water, and menhaden in addition in brackish water. In early December, a huge kill of menhaden in which literally millions of fish died, had occurred in the brackish water of the delta. Individuals of other species were seen or reported but not in significant numbers.

Channel catfish, the principal species affected in fresh water, is commercially important in the Mississippi River. It has been reported that dead and dying fish have been collected by commercial fishermen and sold for human consumption.

The kills have continued throughout the winter of 1963-64, with kills in the Atchafalaya River reported as recently as early March. The kills have occurred sporadically, with no definite pattern as to place of individual kills.

The major portion of the kills seemed to have been from Baton Rouge south. Fish might die upstream from Baton Rouge one week, on the delta below New Orleans another week, and somewhere in between these points at a later date. During the week of December 3, large numbers of dying fish were observed in a navigation canal leading off the Mississippi River immediately opposite Baton Rouge but few dying fish were observed in the river in that area. The discharges from the industrial complex at Baton Rouge did not appear to be related to the mortality pattern.

Personnel of the State of Louisiana had observed dying fish as far upstream as the connection of the Atchafalaya River with the Mississippi

River near the Mississippi State line. Fish kills were observed in the Atchafalaya River. No mortality was observed in the Bayou Lafourche (beginning at Donaldsonville, 2 miles southeast of Baton Rouge) which receives almost its entire flow as water pumped from the Mississippi River.

As in previous years, the water chemistry and animals other than fish appeared normal and unaffected. When the fish began dying in November, the water temperature was 20° C. and there had been no sudden temperature decline immediately preceding the onset of mortality. The mortality rate was reported to increase with the onset of a cold period in late December.

The symptoms of dying fish were the same as in previous years; the fish were upside down at the surface, not gasping air, had hemorrhagic areas around mouth, fins, and on internal organs, and the alimentary tract was distended with gas or liquids. All fish appeared to be in good condition with much internal fat but the alimentary tract contained no food material, either digested or undigested. All dying fish appeared distended with gas so that they had difficulty in submerging. When they were excited or placed under stress, they would go into convulsions or have a convulsion-like response and many would die. Stresses placed on the fish, such as the wake of a tug, would cause individuals to surface, convulse, and often lose equilibrium. At this stage, the fish could be readily netted but often would swim downward after a period of time if not disturbed. Catfish have been observed to expel air at this stage and then appear to swim away normally. Specimens that had expelled air and subsequently were placed in holding tanks, however, died after a short period.

INVESTIGATION INTO CAUSE OF THE FISH KILLS

Various causative factors which might have been responsible for the fish kills were investigated. These investigations included:

1) Environmental Conditions - Adverse environmental conditions resulting from pollution, such as depletion of dissolved oxygen or temperature increases, have been eliminated as causes of the kills based upon field observations and water quality measurements during the kills. The fish kills could not be related to river flows, or known discharges of municipal or industrial wastes.

2) Parasitic or Bacterial Diseases - During the initial fish kills in the winter of 1960-61, the U. S. Fish and Wildlife Service laboratories tested for fish pathagens and parasites, and reported negative results. During the 1963-64 kill, it was established by Drs. Snieszko and Hoffman of the U. S. Fish and Wildlife Service that the kill was not due to parasitic or bacterial disease.

Personnel of the Public Health Service's Division of Environmental Engineering and Food Protection found no evidence of botulism.

Division of Water Supply and Pollution Control personnel fed tissues of dying catfish to healthy catfish to see whether a viral or bacterial disease could or would be transmitted; this investigation yielded negative results.

3) Toxic Substances - Examination of dead fish indicated the effects of a toxicant, as revealed by white blood cell counts and microscopic examination of the kidney.

Analysis of certain enzymes in the brain tissue indicated that death was not due to organic phosphorous insecticides. An analysis of tissue for metals, which was accomplished by emission spectographic analysis, also gave negative results.

Several simultaneous experiments were conducted to detect the presence of toxic materials. Extracts of the mud taken from the area of the fish kills, when placed in aquaria containing healthy fish, resulted in death. Extracts of the tissues of dying fish, when dissolved in the water of the aquaria, also resulted in the death of healthy fish. In both cases death occurred with the same symptoms as those observed in Louisiana.

Immediate steps were taken to identify common substances in the extracts from the mud, from dead fish obtained in Louisiana, and from the fish killed experimentally in the laboratory. Rigorous analysis were made independently by five teams of investigators. Four of these were within the Public Health Service, (Divisions of Water Supply and Pollution Control and Environmental Engineering and Food Protection) and one was by the Monsanto Research Laboratories of Dayton, Ohio. These examinations--by gas chromotography--indicated the presence of of endrin in all extracts and the presence of dieldrin and other chlorinated hydrocarbon insecticides in most. Two teams of investigators were also able to identify endrin by paper chromotography.

At least three materials were found in the blood of every dying fish taken from the Louisiana area. Two of these are unknown (hereafter called X and Y), and the third has been identified as endrin. The ratio X,Y/endrin in general, has been consistent in all fish from the river (living and dead) that have been analyzed. For this and other reasons, it is believed that these two materials are endrin-associated products.

Endrin has been found in extracts of the following tissues from dead or dying fish: muscle, blood, liver, gill, intentine, kidney, spleen, fat and eggs. Although specific concentrations have not yet been computed, estimated ranges (in micrograms per gram of tissue, wet-weight) are: muscle, ½ to 1; liver 1 to 2; and fat and eggs, 6. Blood ranges were computed as from 0.12 to 0.56. (Note: The equivalent values in parts per billion are as follows: muscle, 500 - 1,000; liver 1,000 - 2,000; fat and eggs, 6,000; blood, from 120 to 560.)

In addition, to endrin and compounds X and Y, DDE and DDT have been found in varying amounts in all fish examined including all control fish from numerous locations. The presence of dieldrin has been haphazard and in greatest quantity in living fish from the Louisiana State Penitentiary Lake at Angola, not directly connected to the Mississippi River by surface water.

EXPERIMENTS WITH ENDRIN

A series of tests was conceived and executed to determine whether endrin in the blood of dead fish taken from the Mississippi River was present in lethal amounts. Endrin was chosen because its presence in every dead fish examined made it a likely suspect. A detailed report describing these experiments as well as some of the preceding investigations is attached as Appendix A.

The investigations with endrin required three sets of determinations. First, the amount of endrin in the bloodstream of fish killed under test conditions was determined; second, the amount of endrin in apparently healthy fish taken from the Mississippi River was determined; and third, the amount of endrin in the bloodstream of fish that had

died in the Mississippi River was determined. The purpose of these three determinations was to establish whether or not the blood concentrations of endrin in dying Mississippi River fish represented lethal concentrations of this compound.

The blood samples taken from dying channel catfish in the fish-kill area in December ranged from 0.41 to 0.56 micrograms per gram (410 to 560 parts per billion) of blood. Only three blood samples from approximately six to eight channel catfish were available for analysis The blood from approximately 20 living channel catfish taken from the water of the Mississippi River in January and February, in places where no kill was occurring, were also analyzed for endrin, and the blood levels ranged from 0.08 to 0.17 micrograms per gram (80 to 170 parts per billion)

The blood concentration of endrin in dying buffalo, shad, and bullhead catfish from the river, has been found to be from 0.12 to 0.22 micrograms per gram. (120 to 220 parts per billion) Approximately 20 fish were analyzed. Nine living buffalo collected from the river in early February 1964, had no measurable amount of endrin in the blood.

In the laboratory experiments, catfish were exposed to endrin and the blood concentrations of endrin at death were measured. Channel catfish obtained from the fish hatcheries in Arkansas were used for the experiments.

Known amounts of endrin were introduced into the experimental water, in concentrations ranging from 10 parts per billion to 0.25 parts per billion. (There was a reduction in these initial concentrations, due to absorption by bacteria and fish, and the actual concentrations over the exposure period are not known).

Tests are still in progress at concentrations as low as 0.06 parts per billion to determine possible delayed mortality. One of the theories which has been advanced for the seasonal pattern of the kills is that the fish build up relatively high concentrations of endrin in their fatty tissues over a period of time. During the winter, when the fish do not feed extensively, these fatty tissues are reabsorbed and the accumulations of endrin may be released into the blcod at lethal levels. This theory is now being tested by the delayed mortality experiments.

The experiments showed that the concentration of endrin in the blood of dying channel catfish exposed in the laboratory to various water concentrations of endrin is slightly less or equal to the blood concentration of endrin found in the dying channel catfish taken from the Mississippi River. Secondly, it was determined that the concentration of endrin in the blood of living channel catfish exposed to endrin in the water for periods up to 21 days and from living fish from the river is lower by a factor of two to four than the concentration in the blood of dying fish.

The concentration of endrin in the blood of both living and dying catfish in the Mississippi River was 1000 to 10,000 times greater than concentrations of endrin observed in the river water during August, September, and October of 1963. Careful experiments in which time is an important variable that cannot be compressed will be required to determine if and how the trace quantities of organic poisons observed in the river water build up to lethal concentrations in a fish's blood.

These experiments and related investigations demonstrated that:

1. The levels of endrin in the blood of dying channel catfish from the Mississippi River at Baton Rouge could represent lethal levels.

2. As the lethal levels of endrin in blood in laboratory exposures were comparable to the levels found in dying fish from Louisiana; this indicates that the unknowns, X and Y, although always associated with endrin, were not contributing significantly to the death of the river fish.
3. The symptoms of dying channel catfish from the river and from laboratory exposures are identical. These include gas and liquid distention of the alimentary tract, not heretofore reported in the literature as far as is known.
4. Cellular damage and histological change observed in the kidney of dying channel catfish from Louisiana is consistent with known damage from chlorinated hydrocarbon insecticides.
5. Concentrations of endrin in the Mississippi River, were approaching the lethal level for fishes.
6. No other known insecticide was uniquely related to the dying fish. All positive and negative evidence obtained in the past three months of laboratory studies indicates endrin as the cause of fish mortality and no available evidence refutes endrin as the causative agent.

It therefore appears that endrin was the most likely cause of the fish kill observed in the Mississippi River during the fall and winter of 1963. Because of the many similarities between the fish kills in 1960, 61, and 62, and that which occurred in 1963, it is quite probable that the earlier kills were caused by the same toxic organic materials responsible for the kill in 1963.

TOXICITY OF FISH AND OYSTER SAMPLES TO MICE

Specimens of dead or moribund catfish collected from the Mississippi River at Baton Rouge on December 18, 1963, were tested for toxicity to mice by personnel of the Division of Environmental Engineering and Food Protection.

Vicera of fresh water catfish, (kidney, liver, intestine, spleen) were extracted with acetone and dissolved in vegetable oil. Death in mice was produced by intraperitoneal injection of 0.5 ml. and a 0.3 ml. of the oil extract. These quantities represent approximately 1 gram of a visceral tissue. The larger dose produced death in 5 minutes. Similar extracts of muscle and bone tissue were found to be toxic to mice. The toxicity, however, was 1/20 of that of the vicera. Marine catfish from Dauphin Island Bay extracted in a similar manner produced negative results in mice. Later, the residue of the fish sample material, fed to a small salt water catfish produced symptoms similar to those observed in the fish kills in the Baton Rouge Area. The fish died in approximately 48 hours.

Mouse bioassays were also conducted with oil and water soluble extracts of oysters from Grand Bay, Quarantine Bay, American Bay, and other shellfish growing areas. These experiments produced no deaths in mice.

A summary of these experiments is presented in Table A on the following page.

Definitive chemical analyses of the fish and oyster tissues involved in these experiments are not yet available.

A monitoring program is now being developed to maintain surveillance of several shellfish producing areas along the Gulf Coast. Some of these areas are influenced by flows from the Mississippi; other areas are not influenced by Mississippi flows but receive other agricultural drainage.

BIOASSAY OF FISH AND SHELLFISH - MISSISSIPPI RIVER

TABLE A

DATE	SPECIES	SOURCE	TYPE OF ASSAY	RESULTS
12/18/63	Catfish	Mississippi River Baton Rouge, La.	Acetone - oil extract of viscera - intraperitoneal injection in mice	Death on injection of equivalent of 1 gm. of visceral material
			Ether - oil extract of flesh and bone injected intraperitoneally in mice	Death on injection of equivalent of 20 gm. no death - 10 gm.
			Feeding 15 gm. body tissue to salt water catfish	Death in 48 hours Symptoms similar to Mississippi River kill
1/7/64	Oysters	Quarantine Bay, La.	Ether - oil extract of homogenized whole meats intraperitoneal injection in mice	No toxicity
	Oysters	Adams Bay, La.	As above	No toxicity
1/8/64	Oysters	Grand Bay, La.	As above	No toxicity
1/30/64	Oysters	Quarantine Bay, La.	As above	No toxicity
1/31/64	Oysters	Grand Bay, La.	As above	No toxicity
2/1/64	Oysters	American Bay, La.	As above	No toxicity
3/7/64	Oysters	American Bay, La.	As above	No toxicity
	Oysters	Bastions Bay, La.	As above	No toxicity
3/10/64	Oysters	Adams Bay, La.	As above	No toxicity
3/10/64	Oysters	Bastions Bay, La.	As above	No toxicity
	Oysters	Skipjack Bay, La.	As above	No toxicity

PRESENCE OF ENDRIN IN OTHER ANIMALS

1. Ducks

During the fall and winter migratory season in 1963, there have been periodic observations of individual ducks falling from flying flocks in Louisiana. The Assistant Director, Louisiana Wildlife and Fisheries Commission has estimated the total observations of dead ducks as 100 to 150.

One lesser scaup duck dying in this manner was collected in Louisiana in February and examined for pesticides. Extracts of blood from the heart of the duck were analyzed. The results were as follows: 970 parts per billion dieldrin; 870 parts per billion endrin; 420 parts per billion DDE and DDT; and unidentified peaks corresponding to the unknown compounds X and Y in fish.

2. Shrimp

Although no reports of shrimp mortality have been received, shrimp from the delta region of the Atchafalaya River were collected and analyzed. The following materials were found: 250 parts per billion dieldrin; 360 parts per billion endrin; 90 parts per billion DDE and DDT; 10 parts per billion heptachlor epoxide, unknown compounds X and Y, isodrin or aldrin; and unknown, possibly methoxychlor.

WATER SAMPLING

A number of analyses of raw waters and treated water supplies from the Mississippi River have been made in connection with investigations of the fish kills.

1) Raw Water

During the first week in December, four water samples were collected near the bottom of the river above and below St. Louis, Missouri. These samples were shipped in special containers to the Basic Data Water Quality Laboratory at the Sanitary Engineering Center. Dieldrin was present in all four samples, but endrin, if present,was in a concentration less than .001 part per billion (one part per trillion). Other halogenated substances, not yet identified, were also present. The results of these analyses are shown in Table B.

Carbon filter samples have been collected routinely from a number of points along the Mississippi River since 1957 as a part of the Public Health Service's nationwide water pollution surveillance system. Organic material is concentrated on activated carbon, the organics are then desorbed with chloroform, the amount of organic material is measured, and the resulting extract is stored for future use. Twenty-eight of these extracts of water samples taken from the Mississippi River in the four months of 1963 just prior to the first evidence of the winter kill were available.

Analysis of these 28 samples provided a recent history of average concentrations of endrin and dieldrin in the River. Dieldrin was found at all stations except one from Dubuque, Iowa, to New Orleans, Louisiana. Endrin was not found in quantifiable amounts above Cape Girardeau, Missouri, but it was found there and at all stations downstream. Dieldrin varied from non-detectable to .067 parts per trillion. Endrin

TABLE B

RESULTS OF ANALYSES FOR PESTICIDES OF FOUR GRAB SAMPLES FROM THE MISSISSIPPI RIVER DECEMBER 5, 1963

SAMPLING LOCATION	ANALYSIS Substance/Concentration Parts per billion		REMARKS
A	(Dieldrin	0.048	Five other peaks were present which indicate the presence of at least five additional halogenated compounds. Two of these peaks are suggestive of, but not irrefutably identifiable as, lindane and aldrin.
	(Endrin	< 0.001	
B	(Dieldrin	0.198	
	(Endrin	< 0.001	
C	(Dieldrin	0.030	
	(Endrin	< 0.001	
D	(Dieldrin	0.056	
	(Endrin	< 0.001	

A - 0.25 miles north of Jefferson Barracks Bridge, Mo. Sample taken 1/3 of the way across from the Missouri side at a depth of 16 feet.

B - 1.5 miles north of Alton Dam 26 in State of Missouri 1/3 of the way across from the Missouri shore at a depth of 12 feet.

C - 1.5 miles north of Alton Dam 26 in Madison County, Illinois. Samples taken 1/3 the way across the river from the Illinois shore at a depth of 17 feet.

D - 0.5 miles north of Jefferson Barracks Bridge in St. Clair County, Illinois. Samples taken 1/3 the way across from the Illinois Shore at a depth of 21 feet.

below Cape Girardeau varied from .007 to .214 parts per billion. Other halogenated compounds were indicated by gas and thin layer chromotography, but these substances have not been identified. In general, the number of halogenated compounds was greater at downstream stations.

Results of the analyses of the 28 chloroform extracts are shown on Table C. The results are subject to two conditions: the concentrations are minimum averages and give no indication of peak concentration; furthermore, the efficiency of the carbon-concentration-desorption technique is not accurately known for all of the conditions under which the samples were taken and, therefore, the true average concentration is probably greater than the measured value. The identification of the pesticides in both the grab samples and the carbon absorption samples was corroborated by several analytical approaches.

Identification of these organic materials in minute concentrations in water, as well as fish tissues, has been dependent upon recent break-throughs in analytical techniques. The techniques used in sample analysis are described in detail in Appendix B.

Over 2,500 carbon filter extracts collected from sampling points in the Mississippi Basin since 1957 are now available for analyses utilizing these new techniques. These samples represent a continuous record of past water quality conditions in the basin. A portion of these samples have now been selected for analyses, and work is beginning to develop the historical pattern of pesticide levels in the Mississippi River during this period. It is anticipated that this work will be completed during the next several months.

TABLE C

RESULTS OF PESTICIDE ANALYSES OF CARBON CHLOROFORM EXTRACTABLES FROM CARBON ADSORPTION METHOD SAMPLES FROM NINE NWQN STATIONS, JULY, AUGUST, SEPTEMBER, OCTOBER 1963

NWQN SAMPLING LOCATION	RIVER MILE	SAMPLING DATE 1963	ANALYSIS[a] Endrin	ANALYSIS[a] Dieldrin	REMARKS
			Parts per Billion		
#27-Lock & Dam #3 below St. Paul, Minn.	1757	8/6-8/13	<0.001	<0.001	
		9/3-9/10	<0.001	<0.001	
		10/1-10/8	<0.001	<0.001	Other halogenated compounds were indicated by thin layer and gas chromatography. These substances have not yet been identified. In general, however, the number of halogenated substances was greater at downstream stations.
#26-at Dubuque, Iowa	1549	8/10-8/15	<0.001	0.067	
		9/4-9/17	<0.001	<0.001	
		10/7-10/14	<0.001	<0.001	
#25-at Burlington, Iowa	1369	8/5-8/13	<0.001	0.001	
		9/2-9/19	<0.001	<0.001	
		10/7-10/15	<0.001	0.001	
#24-at East St. Louis, Ill.	1166	8/20-8/26	<0.001	0.003	
		9/17-9/23	<0.001	0.003	
		10/8-10/16	<0.001	0.001	
#23-at Cape Girardeau, Mo.	1020	8/5-8/19	0.007	0.005	
		9/3-9/11	<0.001	0.003	
		10/7-10/21	<0.001	0.001	
#22-at West Memphis, Ark.	726	8/13-8/20	0.019	0.002	
		9/3-9/9	0.026	0.015	
		9/30-10/14	0.214	0.058	
#54-at Delta, La.	433	7/1-7/8	0.022	0.022	
		-	-	-	
		-	-	-	
#21-at Vicksburg, Miss.	431	8/5-8/15	0.020	0.005	
		9/9-9/18	0.020	0.004	
		10/9-10/18	<0.001	0.003	
#20-at New Orleans, La.	105	8/5-8/8	0.074	0.013	
		9/6-9/12	0.082	0.032	
		9/13-9/20	0.083	0.024	
		10/2-10/8	0.060	0.007	
		10/10-10/15	0.134	0.011	
		10/23-10/28	0.054	0.034	

a/ Concentration values are calculated from quantitative gas chromatographic analysis of aromatic fractions of CCE. In the light of the unknown efficiency of carbon adsorption sampling for these compounds, the reported values represent minima; the actual values being equal to or most likely greater than the reported values.

2) Treated Water Supplies

Grab samples of partially treated and finished water from the New Orleans public water supply system were collected on March 5, 1964, and analyzed for pesticides. The sample of finished water collected at the New Orleans Water Treatment Plant contained 0.025 parts per billion of endrin and <0.007 parts per billion of dieldrin. The sample of softened, coagulated, settled, carbonated water (not filtered or chlorinated) collected at the East Jefferson Parish Water Treatment Plant was negative for these organics.

Two carbon chloroform extracts of finished water were also obtained from New Orleans. These extracts had been analyzed for endrin. The first extract represented finished water passed through a carbon column from August 10 to August 24, 1961. The analysis showed that there was at least an average of .006 parts per billion of endrin in the finished water during that period. The second sampling period was from July 28 to August 13, 1963. The analysis showed that during that period, there was an average concentration of at least 0.160 parts per billion of endrin in the finished water. The analysis was confirmed using infrared. This is believed to be the highest average concentration of endrin observed in a finished water supply.

TOXICITY OF WATER SAMPLES

Division of Environmental Engineering and Food Protection personnel conducted a number of experiments on the toxicity of water from the lower Mississippi River and from shellfish growing areas influenced by flows from the Mississippi. Test animals used were brine shrimp and a small brackish water fish, Mollenisia laptima. A summary of these

experiments is presented in Table D on the following pages.

Samples of Mississippi River water collected in January 1964, were found to be toxic to brine shrimp, and fish with gradual reduction in toxicity in successive samples. The toxicity was removed by filtration thru activated carbon. Samples collected in March were found to be not toxic.

Water from several marine areas tributary to the Mississippi River were not toxic to brine shrimp or the test fish, Mollenisia.

PATTERN OF PESTICIDE USE

As an initial step toward determining the sources of pesticide contamination, an assessment was made of the use of aldrin, dieldrin and endrin in a major portion of the Mississippi River Basin. This assessment, made by personnel of the Division of Water Supply and Pollution Control's Pesticide Pollution Project, Atlanta, working with State Agricultural Experiment Stations, is presented as Appendix C.

The data show a total estimated usage of 1,748,000 pounds of endrin in 1963, with the bulk of the use in Mississippi, Arkansas, Oklahoma and Louisiana. There was an estimated total usage of aldrin of 10,616,000 pounds, with heaviest usage in Illinois, Iowa and Missouri and no use recorded in the downstream States. Aldrin is of concern because it is transformed in the soil to its epoxide, dieldrin.

In addition to agricultural usage, at least one producer of these insecticides is located within the basin. A plant producing endrin is located at Memphis.

TABLE D

BIOASSAY OF WATER FROM LOWER MISSISSIPPI RIVER AREA

DATE	SOURCE	TYPE OF ASSAY	RESULTS
12/1?/63	Mississippi River - Baton Rouge, La.	Bioassay - Exposure of brine shrimp to various dilutions of water in aquaria.	Toxic - LC 50 at 65% concentration of River Water.
1/8/64	Mississippi River - Olga, La.	As above.	Toxic - LC 50 at 90% concentration.
1/7/64	Quarantine Bay, La.	As above.	No mortality.
1/8/64	Grand Bay, La.	As above.	No mortality.
1/3?/64	Mississippi River - Olga, La.	As above.	Toxic - LC 50 at 90% concentration.
		Bioassay - Exposure of fish (Mollenisia laptima) to water in aquaria.	100% mortality - 72 hrs. Symptoms similar to Mississippi River fish kills. Carbon filtered sample not toxic.
1/30/64	Quarantine Bay, La.	Bioassay - Brine Shrimp	No mortality.
1/31/64	Grand Bay, La.	Bioassay - Brine Shrimp	No mortality.
3/10/64	Adams Bay, La.	Bioassay - Brine Shrimp	No mortality.
		Bioassay - Fish (Mollenisia)	No mortality - 144 hours.

(Cont'd)

TABLE D

BIOASSAY OF WATER FROM LOWER MISSISSIPPI RIVER AREA (CONT'D.)

DATE	SOURCE	TYPE OF ASSAY	RESULTS
3/11/64	Mississippi River - Olga, La.	Bioassay - Brine Shrimp	Untreated water - No toxicity 72 hrs. Sample concentrated tenfold - No toxicity - 72 hours.
3/11/64	Johnsons Pass, La.	Bioassay - Fish (Mollenisia)	No mortality.
		Bioassay - Brine Shrimp	No mortality.
3/23/64	Mississippi River - Olga, La.	Bioassay - Fish (Mollenisia)	No mortality.
		Bioassay - Fish (Mollenisia)	No mortality.
3/24/64	Eastern Barataria Bay, La.	Bioassay - Fish (Mollenisia)	No mortality.
3/24/64	Redfish Bay, La.	Bioassay - Fish (Mollenisia)	No mortality.

FUTURE COURSE OF ACTION

Although the findings have been highly significant with respect to the cause of the fish kills, they have also highlighted the complex and difficult nature of the Lower Mississippi problem. There have been many cases of fish kills resulting from insecticide use in the past. Usually, however, these have been the result of accidental spillages, careless spraying operations, or heavy rains and run-off shortly after the chemical has been applied. The kills have usually been relatively localized and acute. The particularly alarming aspect of the findings on the lower Mississippi, is that these kills are much more widespread; their long and recurring nature suggests a continuing contamination of the environment.

The current investigations represent merely a start in terms of developing a program of prevention and control. A whole host of questions concerning this problem remain to be answered. These include:

1. More information is needed on the extent and pattern of contamination of the water resources of the lower Mississippi area, including ground waters and public water supplies. Developing this information and keeping it current will require a more complete surveillance system.
2. Continued improvements in analytical techniques will be needed to provide for quick, routine means of detecting and measuring these contaminants at the fractional parts per billion level.
3. Information is needed on the relationship of the pattern of pesticide useage to observed concentrations in the water. It will be necessary to understand the relationships between pos-

sible industrial discharges, application methods, hydrological, geological, climatological, and land use factors, and contamination of water resources.

4. The mechanisms whereby pesticides and other pollutants move through the food chain and the environment into fish must be understood, as must the mechanisms whereby these materials are concentrated and cause toxicity. Determination of chronic and long-term effects on fish and shellfish at various stages of development, and on food chain organisms will be necessary to arrive at safe levels for the preservation of fisheries.

5. A program on the relationship between water pollution and human toxicology and epidemiology should be implemented.

6. Methods of control of pesticides and other organic contaminants must be sought. These may include control at the source through improved methods of application or waste disposal, through developing means of binding these materials to the soil, or through the substitution of biodegradable materials for these presently in use.

Further, the lower Mississippi area receives the drainage from a major portion of the United States, and wastes from a myriad of sources contribute to pollution of this area. Pollution problems in this area are complex, and any program should be designed to provide overall remedial measures.

On the basis of the findings to date, the Secretary of Health, Education, and Welfare has called a four-State enforcement conference under the provisions of the Federal Water Pollution Control Act. The

purpose of the conference will be to seek possible remedial measures which can be taken at the present time, and to delineate the needs for further information. The Public Health Service proposes to initiate an over-all and intensified water pollution control study in cooperation with the States and other Federal agencies and industries concerned, to obtain the information necessary to develop both immediate and long-range remedial measures.

During the interim period the Public Health Service is continuing surveillance of the situation. Arrangements are being made for consultation with State sanitary engineers and municipalities on ways and means for minimizing the amount of pesticides passing through water purification works.

The Division of Environmental Engineering and Food Protection Laboratory at Dolphin Island is running shrimp and oyster samples about twice a month with specific examination for pesticides.

Work is being done to define the sources of pollution. Personnel of the Division of Water Supply and Pollution Control's Basic Data Branch are beginning to outline plans for a more complete monitoring system for pesticides. Work is already underway to develop the historical pattern of pesticide levels in the Mississippi River as a means of understanding the sources of this contamination. Over 2,500 carbon filter extracts collected from sampling points in the Mississippi Basin since 1957 are available for analyses utilizing recently developed analytical techniques. These samples represent a continuous record of past water quality conditions. A portion of these samples have been selected for analyses, and it is anticipated that this information will be available within the next several months.

APPENDIX A.1

"BIOLOGICAL INVESTIGATION INTO CAUSE OF MISSISSIPPI RIVER FISH KILL"

Introduction

The following technical summary of the biological investigation of the Mississippi River fish kill includes all data that have been collected since December 1963 and that are pertinent to the investigation.

Preliminary Investigation

Hematocrits of dying catfish blood were 50 percent of normal, and a rough estimation of white cell counts indicated that these were also low by the same amount when compared to the number of red cells. Histological studies of tissues also revealed vacuolation of the kidney tubules. These observations, coupled with the apparent normal conditions of the river in regard to chemical and physical characteristics and invertebrate populations, led us to conclude that the cause of mortality was due to a toxicant.

Freshwater drum were examined by two individual chemical laboratories immediately upon arrival from Louisiana. One laboratory extracted the tissue with hexane after saponification in alcoholic potassium hydroxide, and another used a mixture of hexane and methanol and subsequent azeotropic distillation. Both laboratories obtained strikingly similar gas chromatograms that indicated the presence of known pesticides and other unidentified peaks.

Concurrently, several chloroform extracts (1) of the bottom muds were collected in the area of the fish kill. The first of these was from one liter of mud and weighed 41 mg. This extract was dissolved in 3 ml of acetone, and then 400 ml of pond water were added and mixed. A control was treated in a similar fashion, except no extract was present.

The water containing the extract killed two young guppies in 48 and 72 hours, and a small bullhead catfish after 48 hours. The fish were not observed at death, hence no description of toxicity symptoms are available. The controls of both species were unaffected.

The above extract was made from wet mud. A second extract (2) made from one liter of dry mud yielded 105.5 mg of extract. This extract was dissolved in 3 ml acetone; and then 1000 ml of water, and along with a control, was cooled to 65°F in order to reduce loss of volatile material. A small (3") bullhead catfish was placed in each, and after 14 days, the fish exposed to the extract began convulsing. On the fifteenth day, convulsions were only one to two hours apart so the fish was sacrificed as was the control. The convulsing fish's hematocrit was 28 percent, the control was 45 percent.

A third (3) extract, made as described for the second extract, and weighing 220 mg, was dissolved in 3 ml acetone and then in 300 ml of pond water. The extract killed the small bullhead in less than 15 hours, again without being observed. Autopsy revealed inflammation of intestines and some gas distention of the stomach. A second bullhead placed in this same extract convulsed after five days and appeared to be near death. It was sacrificed and extracted (3b) by the Schafer method (1963). One laboratory found a similar chromatographic pattern for this extract and for the extract of the mud. Furthermore, the patterns were the same as had been found in the previous extracts from the dying drum tissue. A similar (4) extract from the control catfish showed no peaks except one for DDE.

The unused portions of the extracts (3b) from the bullhead exposed to the chloroform mud extract, and the extract (4b) from the control fish were evaporated to dryness, and 20 ml of pond water were added to each.

One small guppy was placed in each extract, and the extract from the convulsing bullhead produced convulsions in the guppy after 54 hours. Death followed at 72 hours, while the control guppy appeared normal.

At the same time, a similar assay was made on liver extract (5) from a dying channel catfish and on a liver extract (6) from a normal channel catfish (normal catfish was from Louisiana State University Lake). In each case there was the extract from approximately 0.1 g of tissue in each ml of water. The guppy exposed to the extract of the dying catfish convulsed after 6.5 hours and died in less than 20 hours.

Five separate extracts (7) were also made by placing 50 to 100 g of muscle from dying and control catfish in a flask with water. The flask was boiled, and the first 20 ml of distillate were collected. Five milliliters of a very hard spring water were added as a buffer, and five small guppies placed in each. The distillates from the dying fish produced convulsions in assay fish in 4 to 24 hours, depending on the amount of tissue used. Control fish distillates were not toxic.

Thus far, in all of the gas chromatograms from dying fish only endrin and two large peaks of unknown materials were always present. Dieldrin and DDE were sometimes present. By correlation, endrin was implicated as being involved as a toxicant as well as the two unknown materials. Effort was concentrated at this point to get better identification of these three materials. Both laboratories had presumptive identification of endrin by electron-capture gas chromatography, one having used three separate instruments. A third laboratory had found a peak corresponding to endrin by organic chlorine determination. A private industrial research laboratory, using electron-capture gas chromatography, examined liver, blood, kidney, gut, muscle, and gill extracts from normal and dying channel

catfish and found the same pattern for endrin and the two unknowns. In addition, because of the specific condition and type of column used, they found two peaks for standard, recrystallized endrin which was provided by one of the SEC laboratories, (instruments in this laboratory gave only one peak) and a double peak in the extracts from dying fish. Two of the laboratories at SEC had also found a paper chromatogram spot which might be endrin.

Infrared identification of fractions obtained from a fraction collector failed due to column bleed, but fractions were collected and assayed for toxicity, using baby guppies as previously described. A blank fraction was collected first. Concentrate of 5 ml of hexane was injected into the chromatograph and the collection vessel was left in place for a period of time, sufficiently long for endrin to elute if it were present. Then an extract from the control catfish muscle was injected, and the two unknown peaks were collected in one vessel, and the DDE-dieldrin-endrin group was collected in another vessel; concentrate from 5 ml was used. Retention time was used to determine when to collect the fractions from the controls. Finally, extract from a dying channel catfish was injected and two fractions were collected. Part of the material eluted from the column was simultaneously passed through the electron-capture detector so that the fraction being collected could be visualized.

The resulting residue in the collection vessels was dissolved in hexane, transferred to a 10-ml beaker, evaporated, 2 ml of pond water added, and 1 baby guppy put in each. The guppy in the endrin fraction from the dying muscle extract convulsed after 6.5 hours and died in less than 14 hours. The other four fish remained normal for six days, and

the test was discontinued. There was the collection from 2.5 g of tissue per 1 ml of water; however, the efficiency of that collection is not known.

This same sequence was repeated using the same procedure except 10 ml of the same extracts were concentrated and injected, and the residue was put in 5 ml of water. The endrin fraction from the dying muscle again produced convulsion; this time in seven hours and death in less than 24 hours. A second fish was placed in this water, and it died in less than 48 hours with convulsions. The other four fish remained normal.

The extract (1/30-1) of dying muscle used for these collections did not have dieldrin present; whereas the extract of the control catfish muscle did have some but it was too low to measure without concentration. DDE was present at approximately 0.08 ppm. In separate tests, it was found that DDE was not toxic in water-saturated solutions. Essentially, these facts pinpointed the toxicant in this fraction as being endrin.

Subsequent work with the collector revealed a time lag between the detector and collector. This did not affect the endrin collection seriously because that fraction was collected for a long period of time. The unknown fraction eluted rapidly, however, and it was possible these materials might not have been collected.

After the lag had been determined, a fraction containing the unknown was collected from 30 ml of dying muscle extract. This, when dissolved in 5 ml of water, produced convulsions in baby guppies in three hours and death in less than 15 hours. Even a 16 to 1 dilution of this extract produced convulsions and death in three days. This 16 to 1 dilution represented the collection from 0.36 g of tissue per ml of water. Again, efficiency is not known; but a very small aliquot had been checked on the gas

chromatograph and was found to contain the two unknown peaks. The aliquot was not checked adequately, however, for the presence of DDE-dieldrin-endrin.

Subsequently, the efficiency of collection was established using aldrin standards and found to be 10-20 percent. Fractions were then collected from 10 ml aliquotes of dying muscle extract; the two unknown peaks were collected in Fraction No. 1, and the DDE-dieldrin-endrin in Fraction No. 2. DDE was present at a concentration of 0.08 ppm and there was a trace of dieldrin. The fractions were dissolved in 1 ml of hexane, and 5 µl of each were chromatographed completely. Fraction No. 1 had none of Fraction No. 2 material present, but Fraction No. 2 had a very small quantity of the second unknown material of Fraction No. 1.

Each fraction in hexane was transferred to a 10-ml beaker, the hexane allowed to evaporate, and 5 ml of pond water added to each. One baby guppy was added to each fraction and after 3.5 hours, the guppy in the endrin fraction was convulsing and was dead after six hours. A second guppy subsequently was put in the fraction, and it convulsed and died in a similar period. The endrin concentration based on the rechromatographed 5 µl aliquot was 40.0 ppb in the 5 ml of water, and the toxicity was comparable to known concentrations of 40.0 ppb.

The fractions from a second 5 ml aliquot of the same extract were collected the following day and assayed for toxicity in a similar manner. This time, the #2 fraction contained a little more of the fraction #1 material than previously. The guppies in the DDE-dieldrin-endrin fraction died in approximately six hours and convulsed after three hours.

The fish in the #1 fractions had not died after four and three days respectively. Tests of longer duration are not dependable in such small volumes.

Greatest reliance should be placed on the last two tests because more experience had been gained in the technique of fraction collection. In addition, these fractions were carefully chromatographed, and it was shown that no DDE-dieldrin-endrin was present in the #1 fraction. In the one test in which fraction #1 was toxic, it was suspected this fraction was contaminated with a toxic material, perhaps some endrin from a preceeding injection, and, therefore, gave a positive result.

This time, experimental exposures of channel catfish to endrin were begun and analyses of blood collected from dying fish from the Mississippi River were initiated. Also, additional living fish and blood samples were obtained and frozen early in March for shipment to the Sanitary Engineering Center. These fish were from the Atchafalaya Basin and no kill was occurring at the time the living fish were collected. Blood samples were collected in the field as described below for laboratory exposures.

Experimental Exposures

Channel catfish, weighing 0.5 to 1.0 pound, were obtained from a fish hatchery in Arkansas. The water supply was from a well and tests indicated no insecticide residues in the blood except some background DDE or DDT.

Exposures were made in stainless steel troughs 6' x 1' x 1', with a water depth of 6 to 7 inches and a flow rate of 150 ml/min. The endrin (Technical grade endrin--91% assay--obtained from Shell Chemical Co.) was dissolved in acetone, 0.4 mg/cc and then this stock was added to the final solution which was 50 percent acetone and 50 percent water. The final stock solution was 400, 1600, or 4000 ppb endrin, depending on

the range of desired concentration. The 50 percent stock was added to the water at the rate of 1 ml per 400 ml of water for the highest concentration, and dilutions of this 400 ml were made for lower concentrations (Unpublished method). All equipment was glass except for plastic connections between glass tubing. The resulting acetone concentration in the test water was at least one-eighth of the TL_m (Median Tolerance Limit) value and probably was much lower because of rapid bacterial decomposition. As of this date, we have not been able to measure the concentrations of endrin in the test chambers satisfactorily, but they probably are much lower than the nominal concentrations.

The water was a pond water that was made by running carbon-filtered tap water into a 100,000 gallon outdoor concrete pool and then back into the laboratory. The retention time in the pool was 90 days and the water contained many forms of zoo- and phytoplankton. Its pH was 7.8 to 8.0, and hardness as $CaCO_3$ was 100 ± 10 mg/l. The DO (Dissolved Oxygen) in the troughs could not be maintained above 3 mg/l due to decomposition of the acetone in the water. This DO level does not represent an adverse environmental condition for catfish, and the fish did not show any oxygen stress symptoms other than increased breathing rate. Only fish displaying convulsions and loss of equilibrium were used for analyses. Three fish died without typical symptoms, but this is expected since they were not acclimated the usual four weeks before testing.

Blood was obtained by wrapping the fish in a towel to absorb water, and then the caudal peduncle was cut off and the blood was dropped into a glass beaker and frozen. Extractions were made by weighing a 3 to 5 g sample of blood in a 50-ml beaker and adding 20 ml of alcoholic potassium hydroxide (36 ml of 100 percent ethyl alcohol, 4 ml water, 10 g of potas-

sium hydroxide). The sample was heated in a waterbath for 15 minutes or until black color appeared. The sample was cooled to room temperature, transferred quantitatively to a 125-ml separatory funnel using distilled water rinse, and then 10 ml of hexane was pipetted into the funnel. Samples were shaken four minutes, allowed to separate, and then the water phase was drawn off, discarded, and the hexane was run into a glass vial. Only 70 to 80 percent of the hexane was recovered. The extracts were stored in a refrigerator to reduce evaporation until analyses were made.

A Jarrell-Ash chromatograph was used for the blood analysss with an electron-capture detector, a 1/4' x 6' aluminum column containing Dow 11 and Epon 1001 on Fluoropak at 180°C. Fifty-microliter samples were used and samples were run interspersed with standards so that approximately six samples and 4 to 5 standards were run each day. Peak height was used for the standard curve, and the curve was linear from 1 to 12 nanograms.

Blood samples were extracted in groups of 6 and alternate groups contained a duplicate sample, one of the duplicates containing an intentionally added 1.6 µg of endrin. The extracts were obtained daily and sent to one of the chemist's laboratories with only a sample number and tissue weight. On several occasions, samples were intentionally or unintentionally analyzed twice, and reproducibility was with 5 percent or less variation.

Table I lists the results of blood analyses for experimental exposures; Table II lists the recovery percentage of spiked samples; Table III lists the blood concentration of dying river fish; and Table IV lists the blood concentration for living fish from the Mississippi River. The

dying channel catfish exhibited symptoms identical to those observed in the dying river fish; namely convulsion, loss of equilibrium, and gas and liquid distention of the alimentary tract. Some hemorrhaging around fins, mouth, and on the viscera was also present.

Discussion

Longer exposures were not deemed necessary because mortality would not be expected based on other work (Mount, 1962). Mortality usually occurs within the first few weeks of exposures. It is concluded, therefore, that the blood levels for experimental exposures exceeding one day in duration would not be different significantly from any longer term exposure.

The values given for blood in the table for living fish at 1.2, 0.6, 0.5, and 0.25 for 4 and 21 days are lower by a factor of 2 to 4 than the lethal values observed. A water concentration of 1.2 ppb would have been lethal upon continued exposure. The values for living fish compared to dying fish indicate that there is a pronounced increase in the endrin concentration of the blood at or near death. Similar observations have been made on several heavy metals at this laboratory.

The blood samples taken from dying channel catfish in the fish-kill area ranged from 0.40 to 0.56 ug per gram (ppm) of blood. Only three blood samples from approximately six to eight channel catfish were available for analysis. The endrin concentration of the muscle of these fish (approximately 0.5 to 1.0 ppm) was similar to the endrin concentration of 10 to 12 different dying channel catfish, and since the muscle is not a primary storage area (Mount, 1962), it can be assumed that the blood levels of <u>all</u> these fish would have been in the 0.40 to 0.56 ppm range.

The blood samples from 34 living channel catfish taken from the water of the Mississippi River in January and February when no kill was occurring, were also analyzed for endrin and the blood ranged from 0.04 to 0.21 ppm. These values are lower by a factor of 2 to 4 than the lethal levels from laboratory and field specimens. Also, some of these fish may have been suffering from endrin toxicity but had not died.

The blood concentration of endrin in dying buffalo, shad, and bullhead catfish from the river has been found to be from 0.12 to 0.22 ppb. Approximately 20 fish were analyzed. Nine living buffalo collected from the river in early February 1964, had no measurable amount of endrin in the blood.

The concentration of endrin in the blood of dying channel catfish exposed in the laboratory to various water concentrations of endrin was found to be slightly less or equal to the blood concentration of endrin found in the dying channel catfish taken from the Mississippi River. Secondly, the concentration of endrin in the blood of living channel catfish exposed to endrin in the water for periods up to 21 days and of living fish from the river is lower by a factor of 2 to 4 than the concentration in the blood of dying fish.

There are at least three materials that have been found in the blood of every dying fish taken from the Mississippi River. Two of these are unknown (hereafter called X, Y), but possibly toxic, and the third has been identified as endrin to the satisfaction of the analytical chemists at the Sanitary Engineering Center. In addition, five separate laboratories have found a substance with a gas chromatographic retention time equal to endrin, and two laboratories have confirmed this by paper chromatography.

In addition, DDE and DDT have been found in varying amounts in all fish examined, including all control fish from numerous locations. These levels are consistent with levels found in previous studies at this laboratory. The presence of dieldrin has been haphazard and in greatest quantity in living fish from the Louisiana State University lake, not directly connected to the river by surface water.

The ratio X,Y/endrin (in terms of orders of magnitude) has been consistent in all fish from the river (living and dead) that have been analyzed. These materials have been totally lacking in the above-mentioned live buffalo in which no endrin was found. In fact, the ratio X,Y/endrin is precise enough so that X and Y can be used as a criterion for the presence or absence of endrin. These two materials are endrin associated for these reasons:

1. They are found, always and only, with endrin and in the same approximate ratio.
2. They are distributed among different tissues and species in the same approximate ratio.
3. They are found in the bottom mud in the same approximate ratio.
4. They are chlorinated.
5. Small peaks with identical retention times have been found in a 13-year-old technical endrin formulation (although in a different ratio).
6. They diminish proportionately with the amount of endrin in live fish collected after the kill.
7. The rate of disappearance for these materials and of endrin is different with different species.

8. The lethal levels of endrin in blood in laboratory exposures are equal to or lower than the levels found in dying fish from Louisiana, indicating that the unknowns, X and Y, are not contributing significantly to the death of the river fish.
9. The probability that three unrelated chemicals could distribute themselves in such an identical manner in such diverse conditions seems remote.

Conclusions

1. The levels of endrin in the blood of dying channel catfish from the Mississippi River at Baton Rouge represented lethal levels.
2. The symptoms of dying channel catfish from the river and from laboratory exposures are identical. (These include gas and liquid distention of the alimentary tract, not heretofore reported in the literature as far as is known).
3. Cellular damage and histological change observed in the kidney of dying channel catfish from Louisiana is consistent with known damage from chlorinated hydrocarbon insecticides.
4. Concentrations of endrin in the Mississippi River were higher towards the mouth of the river than farther upstream, and were approaching the lethal level for fishes.
5. No other known insecticide was uniquely related to the dying fish.
6. All positive evidence obtained in the past three months of laboratory studies indicates endrin as the cause of fish mortality and no available evidence refutes endrin as the causative agent. All the efforts to date to establish any other cause for the fish mortality have produced negative results.

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APPENDIX A.

Table I -- Blood concentration of endrin in channel catfish in laboratory exposures.

Sample No.	No. Fish	Water Concentration ppb	Exposure Time	ppm in Blood
3/13-6	1	40.0	2.4 hr., dead	1.40
2/27-5	1	40.0	2.4 hr., dead	1.98
2/27-1	1	10.0	5 hr., dead	1.20
2/27-2	1	10.0	5 hr., dead	0.70
3/9-11	1	10.0	6 hr., dead	0.95
3/9-10	1	10.0	6 hr., dead	0.86
3/9-9	1	10.0	7 hr., dead	0.89
3/5-1	1	5.0	32 hr., dead	0.31
3/4-1	1	5.0	33 hr., dead	0.46
3/19-3	1	5.0	33 hr., dead	0.50
3/4-4	1	4.0	48 hr., dead	0.36
3/3-4	1	4.0	48 hr., dead	0.26
3/4-3	1	2.5	48 hr., dead	0.27
3/3-3	1	2.5	48 hr., dead	0.31
3/3-5	1	2.5	70 hr., dead	0.34
3/5-2	1	1.6	13 days, dead	0.39
3/11-10	1	1.2	96 hr., alive	0.23
3/3-6	1	1.2	96 hr., alive	0.16
3/9-8	1	1.0	15 days, dead	0.44
3/9-12	1	0.6	96 hr., alive	0.10
3/5-3	1	0.6	96 hr., alive	0.12

APPENDIX A.

Table 1 (Con'd.)

Sample No.	No. Fish	Water Concentration ppb	Exposure Time	ppm in Blood
3/13-2	1	0.5	21 days, alive	0.13
3/13-3	1	0.5	21 days, alive	0.14
3/13-5	1	0.25	21 days, alive	0.10
3/13-4	1	0.25	21 days, alive	0.13
4/2-4	3	1	21 days, dead	0.24
4/2-5	1	1	9 days, dead	0.42
4/2-6	1	1	11 days, dead	0.23
3/11-7	1 Buffalo Ohio River	2.0	48 hr., dead	0.22

APPENDIX A.

Table II -- Spiked samples of blood.

Sample No.	Percent Recovery
3/13-1	103.8
3/11-3	96.3
3/9-1	89.0
3/5-4	88.0
3/3-1	93.8
3/27-4	Spilled
2/22-5	60.0
2/10-6	100.0
4/2-2	84.3

APPENDIX A.

Table III - Blood concentration of endrin in dying Mississippi River Fish

Sample No.	No. Fish	Species	ppm Endrin in blood
2/19-3	1	Channel catfish	0.41
2/20-4	4-6	Channel catfish	0.40
1/31-6	1	Channel catfish	0.56
2/19-4	1	Bullhead	0.16
2/20-3	1	Blue catfish	0.26
2/20-1	1	Buffalo	0.14
2/19-6	1	Buffalo	0.12
2/19-2	Approx. 10	Shad	0.22
2/19-1	Approx. 10	Shad	0.15

APPENDIX A.

Table IV -- Blood concentration of endrin in living Mississippi River Fish

Sample No.	No. Fish	Species		ppm Endrin in blood
3/11-8	6	Channel catfish		0.14
3/11-9	7	Channel catfish		0.21
3/11-12	8	Channel catfish		0.20
3/11-6	3	Channel catfish		0.11
3/11-5	6	Channel catfish		0.04
3/9-2	1	Channel catfish		0.17
3/9-3	1	Channel catfish		0.08
3/9-4	2	Channel catfish		0.08
3/5-6	1	Channel catfish		0.09
3/11-11	7	Bullhead		0.06
3/11-4	3	Bullhead		0.05
3/11-2	2	Buffalo		None
3/9-5	3	Buffalo		None
3/9-6	3	Buffalo		None
2/22-1	4	Channel catfish	LSU Lake	None
2/22-2	20	Sunfish	Bayou Pidgeon	None
2/22-3	-	Channel catfish	Lake Kilarney	None
2/22-4	-	Channel catfish	Bayou Pidgeon	<0.08
2/22-6	2	Channel catfish	Arkansas Hatchery	None
2/27-3	2	Channel catfish	Arkansas Hatchery	None
3/4-2	1	Channel catfish	Ohio River	None

APPENDIX B

ACTIVITIES OF THE WATER QUALITY SECTION, BASIC DATA BRANCH, IN RELATION TO THE ANALYSES FOR PESTICIDES IN WATER

I. BACKGROUND

The surveillance of surface water for pesticide contamination began in 1957. Carbon filter samples, through which 5,000 gallons of surface water are passed, have been collected routinely from a number of points along the Mississippi as a part of the Public Health Service's nationwide Water Pollution Surveillance System.

In earlier years the aromatic fraction of the chloroform extract of these carbon filter samples was subjected to infrared spectrophotometric examination. Identifications of dieldrin and DDT were made in occasional samples. In 1961 the identification and measurement of pesticides in the aromatic fraction was refined both qualitatively and quantitatively by employing electron capture gas chromatography as a supplement to infrared analysis. In 1962 a capability of microcoulometric titration gas chromatography was added to the analytical scheme. The use of these two forms of gas chromatography has made it possible to attain excellent levels of sensitivity and a high degree of specificity in identification. In 1963 a format employing thin layer chromatography was developed which permits the resolution of the several chlorinated

hydrocarbon insecticides contained in the aromatic fraction into distinct spots on silica jel. The extracts of these spots, freed from the normally interfering background material, are far more amenable to definitive gas chromatographic analysis.

With these analytical tools in operational state, a pilot program for grab sample work was begun in 1963 at four stations on the Missouri River. These samples are analyzed by liquid-liquid extraction of a litter of water, thin layer chromatographic separation of the pesticide components in the extract, and gas chromatographic analysis of the extracts of the thin layer spots.

II. CURRENT WORK IN PESTICIDE SURVEILLANCE

A. Carbon Filter Samples

Carbon filter samples are received on a monthly basis from 95 of the Water Pollution Surveillance System's 128 stations. These stations are located on the major rivers and Great Lakes of the nation and, in general, have provided and will continue to provide intelligence on the kinds of pesticides present in surface waters.

The chloroform extracts are obtained from monthly carbon filter samples from 95 stations. Alternate monthly samples are subjected to detailed analysis for chlorinated hydrocarbon pesticides. In this analysis the aromatic fraction of the chloroform extract is separated into five sub-fractions by thin layer chromotography. These sub-fractions are analyzed with both electron-capture and microcoulometric titration gas chromatography. Infrared spectra are obtained from the aromatic fraction.

Because the efficiency of the carbon filter is not accurately known for the large number of chlorinated hydrocarbon pesticides, the concentration values obtained with this method for each of the pesticides can be considered only as a minimum; the true concentration value being equal to or most likely greater than the value obtained with this method. Nevertheless, the relatively large samples (1,000 to 5,000 gallons) permits important collaborative analytical work which is usually not possible with smaller grab-type samples. Moreover, 5,000 samples collected over the past six years represent a potential historical review of pesticide pollution of surface water on a national scale when they are subjected to new, more specific, more sensitive methods of analyses now developed and available.

B. Grab Samples

These samples are shipped in glass bottles encased in expanded polystyrene cartons. Analyses performed make use of liquid-liquid extraction, thin layer chromatography, and the two types of gas chromatography previously described. It is intended to expand this grab sampling program to areas in the country where pesticides are employed routinely for agricultural insect and weed control.

III. SENSITIVITY AND SPECIFICITY OF ANALYSES

A. Pesticides Identified and Measured

The Basic Data Branch is currently equipped to identify, and has had considerable experience in the identification of dieldrin,

endrin, DDT, DDD, DDE, aldrin, lindane, heptachlor and heptachlorepoxide. The Branch's present equipment will also permit the identification and measurement of the organic phosphorous type of pesticide. The extension of activities into this field, however, will require additional personnel, equipment, or adjustments in current surveillance activities for chlorinated hydrocarbons.

B. Specificity

Throughout the development of its pesticide analytical program, but more convincingly demonstrated since its entry into the field of thin layer chromatography, the Basic Data Branch has recognized the desirability for parallel, corroborative identifications of these compounds. The present pesticide identification program with carbon filter samples shows (1) the material is adsorbed upon carbon, (2) desorbed from carbon with chloroform, (3) that the material is either insoluble, not water soluble, not an acid and not a base, (4) is a neutral benzenesoluble aromatic compound, (5) moves on thin layer chromatography in the same fashion as an identified standard, (6) is eluted from a specific gas chromatographic column equipped with an electron capture detector at the same retention time as, and having the same peak geometry as, an identified standard, (7) and is identical to the standard when chromatographed on the microcoulometric titration system in terms of its retention time, peak geometry and degree of chlorination, (8) the infrared spectra of the aromatic fraction does not refute but supports the identification made by gas chromatography. Such corroborative work can provide identification and quantification which is defensible.

The analysis of grab samples, in which a smaller amount of material is available for analysis, is confined to liquid-liquid extraction, thin layer chromatography separation and gas chromatographic examination of both the electron capture and the microcoulometric titration type.

C. Sensitivity

Although the sensitivities for the various chlorinated hydrocarbon pesticides vary because of their differing chemical structure and physical properties, the electron capture gas chromatograph, properly adjusted, can easily detect 0.001 micrograms. The microcoulometric titration gas chromatographic system can detect 0.025-0.050 micrograms. Therefore, thin layer chromatographic-electron capture gas chromatographic identifications of pesticides in liter water samples can be made at the 0.001 parts per billion (1 part per trillion) level. Additional confidence in the identification and quantification is added, however, if the result can be corroborated with microcoulometric titration gas chromatography. The sensitivity for this three-way identification ranges between 0.025-0.050 parts per billion.

Although the numerical results which stem from carbon filter analyses must be considered as minima as previously discussed, the sensitivity in terms of concentration, in the extremely large (5,000 gallons) samples have a potential of reaching down to 0.00000005 parts per billion.

APPENDIX C

USE OF THE INSECTICIDES ALDRIN, DIELDRIN, AND ENDRIN IN THE MISSISSIPPI RIVER BASIN

I. BACKGROUND

Aldrin and dieldrin were developed in 1948 and began to be used by the public about 1950. Endrin was introduced for experimental purposes in 1951 and reached the market several years later. All three are chlorinated hydrocarbon insecticides. Dieldrin is the epoxide of aldrin, and the latter is known to convert readily to dieldrin. Thus, when aldrin is applied for insect control, dieldrin can be recovered proportionally relative to time. Endrin and dieldrin are isomers.

The following usage data were obtained primarily from authorities in the respective State Agricultural Experiment Stations and represent their best estimates. Although rather accurate information is available on whole State bases, proportionalizing these data on a river basin basis was sometimes difficult.

COTTON

The cotton producing portion of the basin includes parts of Alabama, Arkansas, Louisiana, Mississippi, Missouri, Oklahoma, Tennessee, and Texas. Insect pests of primary concern are the boll weevil and the bollworm. Missouri is far enough north so as to suffer only sporadic damage from either of these pests. The number of applications of insecticides per year will vary in other States with the severity of the insect infestations.

In a normal year the farmer has a choice of about 15 basic insecticides prepared in approximately 25 formulations. His choice will depend upon insecticide prices, and also upon the presence of insecticide resistence among his pests that will reduce the effectiveness of certain compounds. Boll weevil resistence to the chlorinated hydrocarbon type insecticides is widespread, and resistence in bollworms is now developing in some areas.

During the first half of the 1950's aldrin and dieldrin were popular among cotton growers, but this usage has declined to relative insignificance.

Endrin came into use about 1952 and is still applied alone or in combination with methyl parathion. Numbers of applications average 8 to 10 per season. Rates of use per acre application in pounds technical were: aldrin 0.25 to 0.75; dieldrin 0.15 to 0.50; and endrin 0.20 to 0.50.

Table 1 contains a summary of estimated 1963 endrin usage on cotton in the Mississippi River drainage basin.

CORN

The corn belt of the United States extends roughly from Ohio through Nebraska and from Minnesota into Missouri. Soil insects, especially the corn root worm, are a problem throughout the entire area. Aldrin, usually incorporated into the soil, is the insecticide of choice, but insect resistence to aldrin is developing, particularly in Nebraska and bordering States, and beginning to force consideration of substitute insecticides (diazinon, thimet, granular parathion). Usually a single appliation of aldrin is made per year at the rate of

0.5 pound per acre. However, some farmers apply the insecticide at 2 to 3 pounds per acre once every two or three years. Table II is a summary of estimated 1963 aldrin usage in the corn belt States.

RICE

In the Mississippi River Valley rice is a major crop in only two States. Rice was planted on 391,000 acres in 1963 in that portion of Arkansas draining to the Mississippi River. Louisiana has 25,000 to 30,000 acres of rice land in this basin.

The principal insect requiring control is the rice water weevil. Aldrin is used as a seed treatment. This practice results in application in the soil of aldrin at 1/4 pound per acre in Louisiana and 3/8 to 3/4 pound per acre in Arkansas. In 1963, 40 percent of the Louisiana rice acreage in this basin was treated and about 2/3 of the total will be treated in 1964. In Arkansas approximately 80% of the acreage has been treated during each of the past three years.

SUGAR CANE

Louisiana is the only State in the Mississippi River drainage system that produces large acreages of sugar cane. Some 87,000 acres are within this basin. Endrin was first used on a small scale in 1958 to control the sugar cane borer, and has been the principal insecticide applied since that time. The recommended application rate is 1/4 pound per acre with 3 or 4 applications needed in a season. All or nearly all acreage requires protection. About 3/4 pounds per acre were needed in 1959 through 1961, but none was required in 1962 and very little in 1963 because the over wintering larvae were decimated by cold weather those years. Resistence to

endrin is now developing so that farmers in 1964 will begin switching to use of Sevin and Guthion.

WHITE-FRINGED BEETLE

The white-fringed beetle is a serious introduced pest throughout the southern and southeastern States. Dieldrin at 3 pounds per acre as a soil surface application or at 1 1/4 pound er acre worked into the soil of cultivated land is the insecticide of choice. No large areas of land have been treated in recent years. Infestations scheduled for treatment in 1964 are small and scattered. Memphis has been treated in places and treatments have been made in the vicinity of New Orleans.

OTHER USAGES

Dieldrin and aldrin at 1 ounce per acre have been used in Oklahoma and perhaps other plains States on thousands of acres for grasshopper control. This practice ended about 1960 on grazing land, but it still may be used on waste land that in the soil bank when needed.

Aldrin is used to a limited extent to control soil insects in wheat. It has been used to control soil insects attacking tobacco, strawberries and sweet potatoes. This usage has been withdrawn beginning in 1964.

Endrin is employed in some States to control mice in orchards although this usage is not recommended because of the hazard to wildlife. It is used to a limited degree to control the sorghum midge, and has been used as a foliar spray on tobacco. The latter use was withdrawn effective in 1964.

A rather unusual use of endrin is on record in Missouri in 1963. An insecticide dealer at Cape Girardeau sold 1750 pounds of endrin that was used as a soil treatment on 16,000 acres of corn in Missouri and Illinois.

Dieldrin is applied in trickling filters at sewage treatment plants to control filter flies. It has some limited usage in production of white potatoes and for Japanese beetle control.

SUMMARY AND CONCLUSIONS

Data concerning usage of the insecticides dieldrin, aldrin, and endrin in a major portion of the Mississippi drainage basin have been assembled. Aldrin is of concern because it is transformed in the soil to its epoxide, dieldrin, that has been reported in river water and fish.

Endrin is employed principally in the lower river valley on cotton and sugar cane, although essentially none was applied to the latter crop in 1962 and 1963. Total endrin usage amounted to an estimated 1,748,000 pounds in 1963. Over ninety-five percent of this quantity was applied in the States of Arkansas, Louisiana, Oilahoma and Mississippi.

Aldrin is used primarily in the corn belt States where in excess of 10 million pounds were applied in 1963. An additional estimated 160,000 pounds was applied on rice land in Arkansas and Louisiana. Aldrin applications are generally made in such a way as to incorporate the insecticides into the soil.

SOURCES OF INFORMATION

The pesticide usage data were obtained from State Extension and Research Entomologists, and from staff members at State Universities with the exception of the information concerning the White Fringed Beetle Control Program of the U. S. Department of Agriculture. That information was obtained from the Southern Plant Pest Control Region headquarters, ARS, USDA, Gulfport, Mississippi. Information concerning the Mississippi levee system was provided by the Corps of Engineers, U. S. Army.

Estimated 1963

Use of Endrin to Control Insect Pests of Cotton in the Mississippi River Basin

State	Acres Cotton in State	Acres Cotton in Basin	Acres Treated Basin	Rate Applied Per Acre	Number Applications	Total Lbs. Endrin/Season
				(.35)	(1.5)	
Alabama	824,000	150,000	113,000	.2 - .5	1 - 2	59,000
Arkansas	1,350,000	945,000	240,000	.3 - .4	6	504,000
Louisiana	525,000	175,000	116,000	0.25	8 - 10	261,000
Mississippi	1,900,000	900,000	180,000	0.2	8 - 10	324,000
Missouri	355,000	355,000	600	0.1 - 0.4	1 - 2	60
Oklahoma			300,000	0.3 - 0.5	5	600,000
Tennessee	550,000	550,000	insignificant			insignificant
Texas	6,000,000	750,000	did not know	.2 - .4	?	?
TOTALS -	11,504,000	3,825,000	949,600			1,748,000

C-7

Estimated 1963

Use of Aldrin to Control Soil Insects of Corn in the Mississippi River Basin

State	Acres Corn in State	Acres Corn in Basin	Acres Treated	Pounds/Acre Single Appl. in Soil	Aldrin Lbs Total
Illinois	8,500,000	8,500,000	4,000,000	1.1	4,440,000
Indiana	6,400,000	5,440,000	326,000	1.0	326,000
Iowa	10,000,000	10,000,000	3,075,000	1.0	3,075,000
Kansas	1,350,000	1,350,000	934,000	0.5	467,000
Minnesota			500,000	0.5	250,000
Missouri	3,340,000	3,340,000	907,000	1.0 - 1.5	1,098,000
Nebraska	5,500,000	5,500,000	800,000	0.5	400,000
Ohio	3,000,000	1,750,000	200,000	1.0	200,000
South Dakota	1,000,000	1,000,000	500,000	0.5 - 1.0	375,000
Tennessee	900,000	900,000	50,000	0.5	25,000
TOTALS -			11,292,000		10,616 000

APPENDIX-D

LETTER REPORT TO LOUISIANA WILDLIFE AND FISHERIES COMMISSION

March 26, 1964

Mr. Robert A. LaFleur, Chief
Wildlife and Fisheries Commission
Division of Water Pollution Control
Drawer FC, University Station
Baton Rouge, Louisiana

Dear Mr. LaFleur:

This will confirm previous verbal reports and discussions concerning the cause of the fish kills in the Atchafalaya Basin and Lower Mississippi River during the fall and early winter of 1963.

After receiving your request for technical assistance, the situation was reviewed at Headquarters of the Division of Water Supply and Pollution Control. Because of the recurring nature of the kill, the wide variety of fish life involved, the extension into both fresh and salt water, and the possible grave water pollution and public health implications, the Division Chief assigned all pertinent available resources of the Division to the task of finding the cause of the kills. In addition, assistance was sought and provided by the Division of Environmental Engineering and Food Protection of the Public Health Service.

In addition, we notified the Bureau of Sports Fisheries and Wildlife, Department of the Interior, and learned that they were carrying out investigations as requested by the State of Louisiana. The Bureau of Sports Fisheries provided us with names of representatives from the Departments of Conservation in Missouri and Illinois. These references proved helpful in arranging for collection of water samples in the vicinity of St. Louis, Missouri, which were sent to the Robert A. Taft Sanitary Engineering Center in Cincinnati, Ohio, for analyses.

Since receipt of your request of November 18, 1963, approximately thirty scientists and engineers of the Public Health Service have participated in the resulting investigations. The resources of the Basic and Applied Sciences Branch, the Basic Data Branch, and the Technical Services Branch were heavily committed. In our opinion, this all out effort was required by the urgency of the situation; and the joint findings of the individual researchers and investigators have justified the effort.

A series of carefully executed time-consuming tests and experiments have demonstrated that the fish kills did not result from botulism, bacterial or viral infections, organic phosphorous insecticides, toxic metals, low dissolved oxygen, an abrupt change in temperature, or other conditions often associated with major fish kills.

The studies have shown the fish kill in the Lower Mississippi River and Atchafalaya River Basin during the winter of 1963 was caused by one or more highly toxic organic chemicals in the chlorinated hydrocarbon category. Although other toxic chemicals were present, the level of endrin in the blood of dying fish was equal to or greater than the concentration found in the blood of dead test fish killed by exposure to endrin in the laboratory. It is, therefore, concluded that endrin was the most likely cause of the fish kill observed in the Mississippi River during the fall and winter of 1963.

Because of the many similarities between the fish kills in 1960, 1961, and 1962, and that which occurred in 1963, it is quite probable that the earlier kills were caused by the same toxic organic materials responsible for the kill in 1963.

The results of our investigations have been made known to other elements of the Public Health Service, the Food and Drug Administration, the Department of Agriculture, Department of Commerce, and the Department of the Interior.

It is recommended that immediate steps te taken in cooperation with the other States in the Lower Mississippi Basin and the States tributary to it to devise water pollution control measures required to protect public health and all other beneficial uses of water including the propagation of fish and wildlife. As our resources will permit, the Division of Water Supply and Pollution Control will participate in cooperative investigations and assist the States in any other appropriate manner.

Sincerely yours,

Keith S. Krause
Chief, Technical Services Branch
Division of Water Supply and
Pollution Control

LOWER MISSISSIPPI RIVER BASIN AREA